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First record of *Mycena plumipes* in Romania, with notes on its presence on pine cones

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Abstract — *Mycena plumipes* was found in Romania on fallen and partially buried cones of *Pinus nigra* and *Picea abies*. The analysis of ribosomal DNA internal transcribed spacer sequences showed that the specimens collected in Romania on *P. nigra* belong to *M. plumipes*. This is the first record of *M. plumipes* in Romania and confirms that it grows on pine cones (as well as spruce cones), based on molecular and morphological data. A detailed description and illustrations of macroscopic and microscopic aspects of the Romanian specimens are given.

Key words — *Basidiomycota*, *Mycena* sect. *Fragilipedes*, *Mycena strobilicola*, habitat

Introduction

Mycena plumipes is a vernal, cone-inhabiting species that belongs to *M.* sect. *Fragilipedes* (Fr.) Quél. (Robich 2006). It was described by Kalchbrenner (1873) under the name *"Agaricus Collybia plumipes"* [i.e., *Agaricus (Collybia) plumipes*]. In his original description, Kalchbrenner did not mention its habitat very precisely, stating the substrate as "ad conos pinuum" (i.e., "on pine cones"), but in the lectotype figure the basidiomata are illustrated on what appears to be a *Picea* cone (rather than a *Pinus* cone). In addition, Kalchbrenner mentioned that *Agaricus plumipes* is accompanied in its habitat by *Agaricus esculentus* Wulfen [≡ *Strobilurus esculentus* (Wulfen) Singer], a species that grows in spring on *Picea* cones (Gulden 1966). Moreau (2003) evaluated these unclear aspects to conclude, based on the iconography and accompanying species, that a cone very similar to one of *Picea* serves as the substrate of *Agaricus plumipes.* Also, Moreau showed that *Agaricus plumipes* is an earlier synonym of *Mycena*

strobilicola J. Favre & Kühner, which also was described from *Picea* cones (Kühner 1938), and went on to propose the combination *Mycena plumipes*.

In Romania, this species was found on fallen cones in plantations of both *Pinus nigra* J.F. Arnold and *Picea abies* (L.) H. Karst. Here we report the first records of *M. plumipes* in Romania and demonstrate the conspecificity of the specimens collected from cones of Norway spruce and black pine using molecular markers.

Materials & methods

Specimens of *Mycena plumipes* were collected in April 2011 and 2012 from fallen and partially buried cones of *Pinus nigra* and *Picea abies*. Fragments from fresh and dried basidiomata were mounted in Congo Red and Melzer's reagent for examination under the light microscope (LM). The specimens were identified using Breitenbach & Kränzlin (1991), Kühner (1938), Elborne et al. (1992), Emmett et al. (2008), Maas Geesteranus (1988), Moreau (2003), Robich (2003, 2006), and Roux (2006). The collected basidiomata were dried and deposited in the Herbarium of the Alexandru Ioan Cuza University, Iași, Romania (I). The description is based on the Romanian specimens collected by the authors.

In order to verify that specimens growing on *Pinus* and *Picea* cones are conspecific (and to confirm the species identity), we targeted the nuclear ribosomal internal transcribed spacer (ITS) region, recently formally proposed as the primary fungal barcode marker (Schoch et al. 2012). The ITS DNA sequences were generated from dried basidiomata using primers ITS1F 5'-CTTGGTCATTTAGAGGAAGTAA-3' (Gardes & Bruns 1993) and ITS4, 5′-TCCTCCGCTTATTGATATGC-3′ (White et al. 1990) and sequenced at a commercial facility (Alvalab, Santander, Spain). The obtained ITS sequences are deposited in GenBank (accession numbers JX297424–297427). Sequences of *Mycena* sect. *Fragilipedes* retrieved from GenBank were aligned with the ClustalW algorithm (Larkin et al. 2007) with a gap opening penalty of 10 and a gap extension penalty of 1. The most appropriate substitution model was selected using a Maximum Likelihood (ML) criterion. The ML analysis with 1000 bootstrap replicates was conducted using the Tamura 3-parameter model with a discrete Gamma distribution (T92 + G) indicated by BIC as the best-fit model. Nucleotide-sequence divergences were calculated using the K2P model (Nei & Kumar 2000). All phylogenetic analyses were performed using MEGA 6.0.5 (Tamura et al. 2013).

Taxonomy

Mycena plumipes (Kalchbr.) P.-A. Moreau, Bull. Mycol. Bot. Dauphiné-Savoie 43(171): 5 (2003) PLATE 1

Basidiomata 40–70 mm high. Pileus 15–30 mm diam., hemispherical, campanulate or conical, with a broad or truncate umbo; finely fibrillose under the lens, hygrophanous, sometimes finely translucently striate at the edge; the colour variable: brown, light brown, ochraceous brown, or greyish brown, margin concolorous or paler. Lamellae 26–35 reaching the stipe (with 3–5

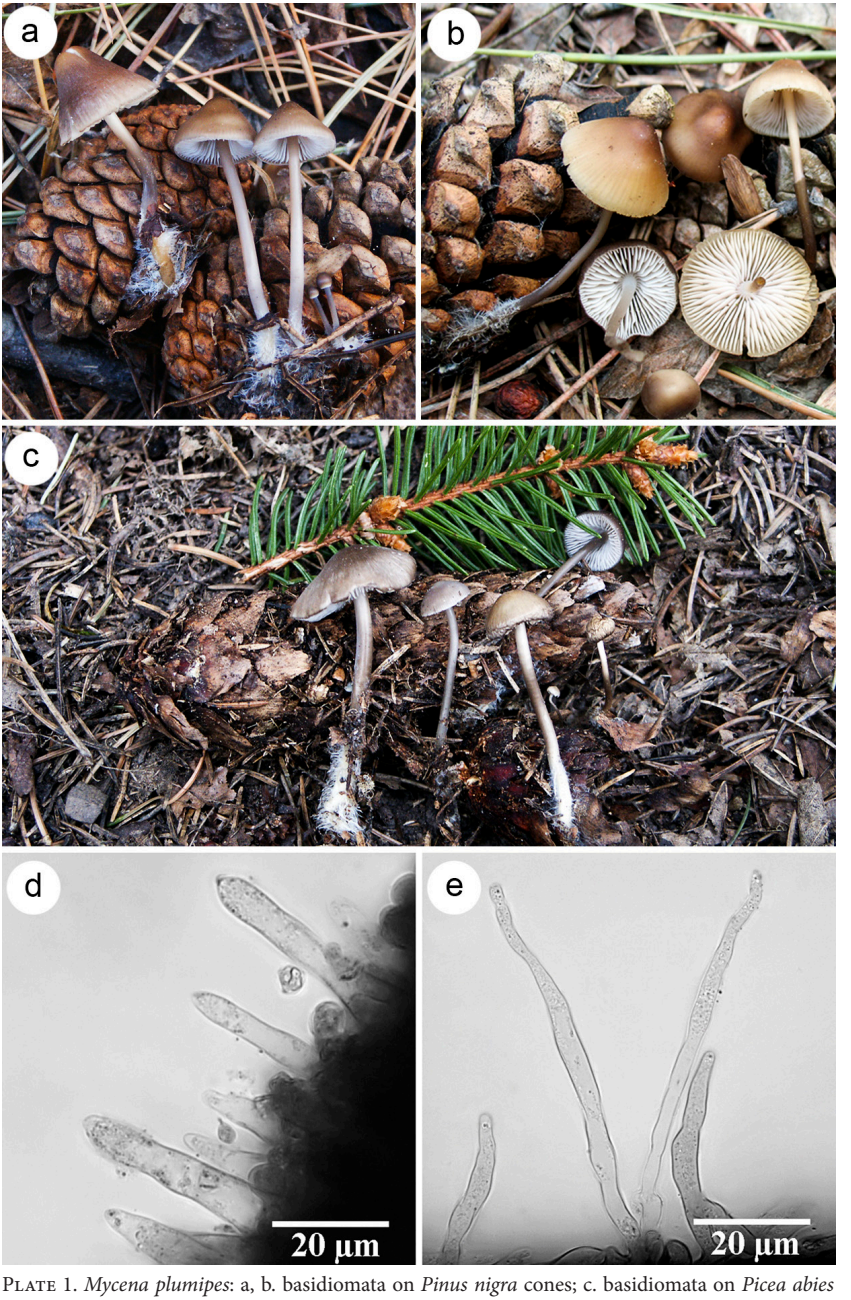

Plate 1. *Mycena plumipes*: a, b. basidiomata on *Pinus nigra* cones; c. basidiomata on *Picea abies* cone; d. cheilocystidia (LM); e. caulocystidia (LM)

intervening lamellulae), ventricose, adnate-emarginate with decurrent tooth, ≤5 mm broad, white, whitish to pale grey, turning to pink with age, edge entire. Spore deposit white. Stipe 30–70 × 2–3 mm, central, cylindrical, straight to slightly curved, equal, hollow, glabrous, grey brown, apex paler to whitish and minutely pruinose, base at the contact zone with the cone covered with coarse, long, whitish fibrils. Context whitish to pale grey. Odor nitrous.

Basidia 25–30 × 6.5–7.5 µm, 4-spored. Basidiospores 7–10 × 4–5 µm, smooth, dacryoid, phaseoliform to ellipsoid, amyloid. Cheilocystidia 30–80 × 9–17 µm, lageniform, fusiform, apically gradually or abruptly narrowed into a longer or shorter neck. Some cheilocystidia have granular refringent secretions on the neck. Pleurocystidia similar to cheilocystidia. Caulocystidia with long cylindrical excrescences, 35–120 × 4–7 µm. Pileipellis hyphae 2–4 µm wide, smooth, rarely with lateral cylindrical excrescences. Clamp connections very rare, present at the hyphae of stipe trama.

Habitat – On fallen and often buried cones of *Pinus nigra* and *Picea abies*, solitary or in small groups (2–5 basidiomata), spring (April).

Specimens examined: **ROMANIA**, **Iaşi County**: Breazu Village, 47°12′45″N 27°31′16″E, 111 m alt., on cones of *P. nigra*, 17.IV.2011, Chinan (I 137185); 2.IV.2012, Chinan (I 137329; GenBank JX297426); near Hlincea Village, 47°07′04″N 27°34′56″E, 104 m alt., on cones of *P. nigra*, 1.IV.2012, Chinan (I 137328; GenBank JX297425); Tomeşti Village, 47°07′47″N 27°42′14″E, 55 m alt., on cones of *P. nigra*, 16.IV.2011, Chinan (I 137184); 1.IV.2012, Chinan (I 137327; GenBank JX297424); **Neamţ County**, near Leghin Village, 47°14′12″N 26°13′58″E, 508 m alt., on cones of *P. abies*, 5.IV.2012, Chinan & Fusu (I 137330; GenBank JX297427).

Molecular analysis – ITS sequences of three Romanian *M. plumipes* specimens were identical (I 137327 and I 137328 from *P. nigra*, and I 137330 from *P. abies*), and the fourth specimen (I 137329 from *P. nigra*) differed by only three transitions (= 0.0038 divergence). The only publicly available sequence of *M. plumipes* (Gene Bank JF908440.1, as *M. strobilicola*) differs in only one nucleotide indel from the sequences of Romanian specimens I 137327, I 137328, and I 137330. In the ML tree, all these sequences are recovered as a highly supported clade, with a bootstrap value of 99 (Fig. 1).

Discussion

Mycena plumipes is widely distributed in Europe (Breitenbach & Kränzlin 1991, Derbsch & Schmitt 1987, Moreau 2003, Robich 2003, Gulden 1966, Gerhold 1989), but it is also mentioned in the Altai Mountains (Singer 1938) and Asiatic Turkey (Solak et al. 1999). It is easy to identify in the field due to its habitat on cones, its nitrous smell, and the whitish fibrils on the base of the stipe.

The Romanian specimens collected from cones of *P. nigra* and *P. abies* morphologically agree with the description provided by Moreau (2003).

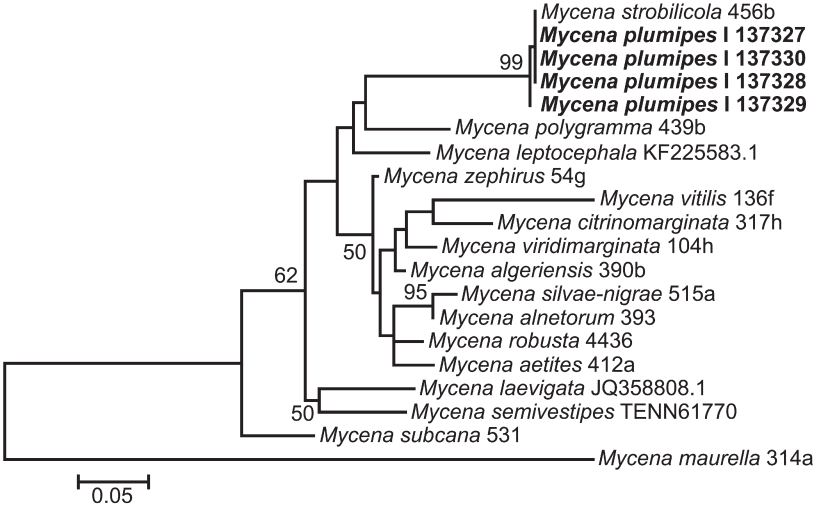

Fig. 1. ML tree based on ITS sequences. Bootstrap values >50, based on 1000 replications, are shown near nodes. Sequences are labelled with voucher number or GenBank accession number. Newly generated sequences are presented in bold font.

In comparing our observations with published data, an important aspect refers to the striation of the pileus, as only some of the specimens from Romania are finely translucently striate at the edge. Moreau (2003) and Roux (2006) describe the edge of the pileus as slightly striate, whereas Kühner (1938), Maas Geesteranus (1988), Breitenbach & Kränzlin (1991), and Robich (2003) state that the pileus is not striate.

The differences between the ITS sequences of *M. plumipes* from Romania are well below the mean intraspecific sequence divergence of 0.025 reported for fungi (Schoch et al. 2012). This confirms the conspecificity of *Mycena* specimens from the cones of *P. abies* and *P. nigra*. The morphologically based identification of the species is further confirmed by the high similarity of our sequences with a sequence available in Gene Bank (accession number JF908440.1) and derived from a specimen collected from Italy, identified as *M. strobilicola* (= *M. plumipes*), in the herbarium of the Museum of Natural History in Venice (Osmundson et al. 2013).

In terms of habitat, in Europe *M. plumipes* is described by most authors as growing on *Picea* cones (Breitenbach & Kränzlin 1991; Derbsch & Schmitt 1987; Emmett et al. 2008; Gulden 1966; Kühner 1938; Maas Geesteranus 1988;

Moreau 2003; Robich 2003, 2006; Roux 2006). However, in some regional species lists it (as *M. strobilicola*) has been reported on pine cones. Gerhold (1989, 1991, 1993, 1994) mentions its presence on pine and spruce cones in Austria, without specifying the species of pine. The species is further reported on pine cones in Asiatic Turkey (Anatolia), specifically on the cones of *Pinus brutia* Ten. (Gezer et al. 2007), *P. nigra* (Gezer et al. 2007, Türkoğlu 2008, Türkoğlu et al. 2007), and *P. pinea* L. (Solak et al. 1999), but without mentioning the diagnosis of this species or highlighting its presence on pine cones. Our results based on molecular analysis and morphology confirm the presence of *M. plumipes* on pine cones as previously reported from Austria and Turkey, an aspect that in the past has been poorly documented and overlooked.

In Romania we found *M. plumipes* in conifer plantations on fallen and buried cones of *Picea abies* and *Pinus nigra*, along with other cone-inhabiting vernal species: *Strobilurus tenacellus* (Pers.) Singer, *S. stephanocystis* (Kühner & Romagn. ex Hora) Singer (on black pine cones), and *S. esculentus* (on Norway spruce cones). From the published data it appears that elsewhere, *M. plumipes* has been found in native forests. Since *Pinus* species are widely distributed, it is intriguing why to date this fungus has been reported on pine cones only from Austria and Turkey.

We found *M. plumipes* in April, specifically in depressions in the soil where the cones gather and the water from melting snow stagnates. The Romanian collections confirm the vernal phenology of *M. plumipes* (Gulden 1966, Kühner 1938, Moreau 2003, Türkoğlu et al. 2007). However, Einhellinger (1977) found this species in Germany in early July. In Asiatic Turkey it seems to have a particular phenology; beside springtime, it has been reported in October (Gezer et al. 2007), November (Gezer et al. 2007, Solak et al. 1999), and December (Türkoğlu 2008, Solak et al. 1999).

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